

APPENDIX J

**PROTEIN RESIDUE ANALYSIS OF ARTIFACTS FROM THE
PUNCHEON RUN SITE**

**PROTEIN RESIDUE ANALYSIS OF ARTIFACTS
FROM THE PUNCHEON RUN SITE**

By

Kathryn Puseman and Linda Scott Cummings
Paleo Research Laboratories
Denver, Colorado

With Assistance From
Curtis Nepstad-Thornberry

THE LOUIS BERGER GROUP, INC.
2300 N Street, NW
Washington, D.C. 20037

Submitted To

U.S. DEPARTMENT OF TRANSPORTATION
Federal Highway Administration

and

DELAWARE DEPARTMENT OF STATE
Division of Historical and Cultural Affairs
Bureau of Archaeology and Historic Preservation

Prepared For

DELAWARE DEPARTMENT OF TRANSPORTATION
Division of Highways
Location and Environmental Studies Office



Eugene E. Abbott
Director of Planning

2004

TABLE OF CONTENTS

		PAGE
I.	INTRODUCTION	J-1
II.	METHODS	J-1
III.	DISCUSSION	J-2
	A. Locus 1	J-2
	B. Locus 2	J-3
	C. Locus 3	J-3
	1. Metate Block	J-3
	2. Feature 30	J-4
	3. Block 3	J-5
	4. Block 6	J-5
	5. General Locus 3	J-5
	D. Locus 5, Feature 99	J-6
IV.	SUMMARY AND CONCLUSIONS	J-7
	REFERENCES CITED	J-17

LIST OF TABLES

	TABLE	PAGE
J-1	Antisera Used in Testing Artifacts from the Puncheon Run Site	J-8
J-2	Provenience Data for Samples from the Puncheon Run Site	J-9
J-3	Positive Results for Samples from the Puncheon Run Site	J-14
J-4	Comparison of Positive Reactions for Artifacts and Soil Controls from the Puncheon Run Site	J-16

I. INTRODUCTION

A total of 83 lithic artifacts from the Puncheon Run Site (7K-C-51) in Dover, Delaware, were submitted for protein residue analysis. Previous studies have shown that protein residues from a tool's surface can be identified using a modified forensic procedure. Site 7K-C-51 contains several discrete activity areas, with diagnostic artifacts datable from Middle Archaic to Late Woodland contexts. Lithic artifacts and soil controls from this site were tested against eight fish antisera and five animal antisera to determine possible fish and animal resources that might have been utilized by the various occupants of the site.

II. METHODS

The artifacts submitted for protein residue analysis were tested using a technique referred to as cross-over immunoelectrophoresis (CIEP or COE). The method for CIEP is based on forensic work by Culliford (1964, 1971), with changes made by Newman (Newman and Julig 1989) following the procedure used by the Royal Canadian Mounted Police Serology Laboratory in Toronto, Canada. Further changes were made at Paleo Research Laboratories, Denver, Colorado, following the advice of Dr. Richard Marlar at the Thrombosis Research Laboratory in the Denver VA Medical Center and the University of Colorado Health Sciences Center.

A total of 75 artifacts were washed using 1-2 milliliters of a 0.02M Tris hydrochloride, 0.5M sodium chloride, and 0.5 percent Triton X-100 solution. Artifacts were placed in an ultrasonic bath for 30 minutes, on a rotating mixer for 30 minutes, and again in the ultrasonic bath for an additional 30 minutes. Eight of the larger artifacts were washed using 20 milliliters of the Tris/sodium chloride/Triton solution. Dirt was removed using centrifugation, and the resulting solution was concentrated to approximately 1 milliliter using a Centricon Plus-20 centrifugal filter device with a 10,000 molecular weight cut-off membrane. Because soils contain compounds such as bacteria and animal feces that can cause false positive results for artifacts buried in the soil, 23 soil controls were also tested. One gram of soil was added to 1 milliliter of the Tris/sodium chloride/Triton solution, and was then refrigerated for several days prior to testing.

CIEP was performed using agarose gel as the medium. The samples were first tested against pre-immune goat serum (serum from a non-immunized animal) to detect non-specific binding of proteins. Non-specific binding is absent if a negative result is obtained. Samples were electrophoresed in Barbital buffer (pH 8.6) for 45 minutes at a voltage of 130. Samples were then pressed and rinsed in 1M saline solution overnight to remove extraneous proteins.

The next morning, the gel was washed, pressed, dried, stained in a Coomassie Blue solution, and then destained. Gels were observed to determine if non-specific binding was occurring. Positive reactions appear as a line of precipitation between the two wells. All samples tested negative against pre-immune serum and were then tested against prepared animal and fish antisera obtained from ICN Pharmaceuticals, Inc. and Sigma Chemical Company, as well as fish antisera raised by Robert Sargeant. Appropriate positive and negative controls were run for each gel. A positive control consists of the blood of each species tested, and a negative control consists of the blood of the species in which the antiserum was raised. Gels were electrophoresed, pressed, washed, dried, stained, and destained as before.

Positive reactions were re-tested with dilute antisera to determine between true and false positives. Antisera were diluted, usually 1:10 or 1:20, to increase specificity of reactions. Positive reactions obtained after this step were reported.

Identification of animals represented by positive results is usually made to the family level. All mammalian species have serum protein antigenic determinations in common; therefore, some cross reactions will occur between closely and sometimes distantly related animals (Gaensslen 1983:241). For example, deer antiserum will react with other members of the Cervidae (deer) family, such as elk and moose, and Atlantic croaker will react positively with other members of the Perciformes order, which include spiny-rayed (percid) fishes.

III. DISCUSSION

Site 7K-C-51 is located in Dover, Delaware, approximately five kilometers from the Delaware Bay. Puncheon Run is a low-order drainage running along the south edge of the site. The site is bounded on the north and east by the St. Jones River, a tidally influenced tributary of the Delaware Bay (Robert Jacoby, Archaeological Field Supervisor, The Louis Berger Group, Inc., personal communication November 1999). A total of 83 lithic artifacts and 23 soil controls from four loci at the site were examined for possible protein residues. These artifacts were tested against American eel, Atlantic croaker, bay anchovy, catfish, gizzard shad, striped bass, trout, weakfish, bear, deer, guinea pig, rabbit, and turkey antisera (Table J-1).

A. LOCUS 1

Fifteen artifacts were submitted for analysis from Locus 1. Two of the 15 artifacts were recovered from the Silo Pit area. This area yielded radiocarbon dates that cluster in the Middle Woodland period, including $1,970\pm 60$ to $1,670\pm 40$ before the present (BP). Sample 98/2/1144 is a Kirk corner-notched chert projectile point found in a flat basin pit (Feature 64) in the Silo Pit area of Locus 1 (Table J-2). This feature is the largest of the "red-ringed" pits, which are believed to have been ancient natural disturbances, although Feature 64 might have been a cultural pit. Radiocarbon dates of $3,600\pm 50$ and $2,830\pm 50$ BP were returned from the feature fill. Sample 98/2/1144 yielded a positive result to deer antiserum (Table J-3). The associated soil control (Sample 98/2/1138) yielded negative results to all antisera tested; therefore, a positive result to deer antiserum for the projectile point suggests that the point was used to hunt a member of the Cervidae family.

Members of the Cervidae family include deer (*Odocoileus* sp.), elk (*Cervus elaphus*), moose (*Alces*), and caribou (*Rangifer tarandus*). Moose and caribou are found mainly in Canada, although moose may be found in the northeastern United States and southwest through the Rocky Mountains to northeastern Utah and northwestern Colorado. Elk are found primarily in the Rocky Mountain region and along the Pacific northwest coast, with great numbers found in Colorado, Wyoming, Montana, and Washington. Elk are reported to have once ranged through most of the United States and Canada, but their numbers decreased as a result of hunting and reduction in habitat from settlement and farming. Mule deer (*Odocoileus hemionus*) are found primarily in the western United States, extending east to Wisconsin and western Texas. Prehistoric ranges may have extended further east. White-tailed deer (*Odocoileus virginianus*) are found throughout most of the United States in a variety of habitats, except for most of California, Nevada, Utah, northern Arizona, southwestern Colorado, and northwestern New Mexico (Whitaker 1980:646-660). The most likely cervid represented by a positive result to deer antiserum for the Puncheon Run Site is white-tailed deer, although moose or possibly elk should not be completely ruled out.

Thirteen artifacts were analyzed from the Buried Plowzone area in Locus 1. Sample 98/2/36.2 is a chert projectile point recovered from Excavation Unit (EU) 257. This sample yielded a positive result to guinea pig antiserum. It is possible that this point was used to hunt beaver (*Castor* sp.), porcupine (*Erethizon dorsatum*), or a member of the squirrel family (Sciuridae); however, no soil control was tested for this artifact. The positive result to guinea pig antiserum might be the result of soil contamination from animal activity in the area. Proteins are present in all body fluids and tissues. Immunological studies on coprolites and modern

animal dung have shown that CIEP will identify which animal produced the feces (Newman et al. 1993). Compounds such as bacteria and iron chlorates can also cause false positive results for artifacts buried in the soil.

Sample 97/58/38 is a jasper late-stage biface from EU 195 that tested positive to both guinea pig and gizzard shad antiserum. No soil control was tested for this artifact. The positive results might represent use of the tool to process beaver, porcupine, or a member of the squirrel family, as well as gizzard shad (*Dorosoma cepedianum*). Alternatively, one or both of these positive results might be the result of soil contamination.

A jasper projectile point (Sample 98/2/79) from EU 266 yielded a positive result to deer antiserum. In the absence of a soil control for this artifact, it is not possible to determine whether the positive result indicates that the point was used to hunt a member of the Cervidae family, such as white-tailed deer, or that the soil has been contaminated by modern deer activity or bacteria.

Sample 98/2/356 is a chert projectile point found in EU 360. Sample 98/2/356S is a soil control for the projectile point that yielded negative results to all antisera tested. The projectile point tested positively to American eel (*Anguilla rostrata*) antiserum, suggesting that it was used to procure the American eel. The American eel is a freshwater eel with small scales embedded in the skin. After reaching sexual maturity, American eel migrate to the Atlantic Ocean to spawn. It is believed that only females ascend rivers, where they may remain for a number of years. American eels are noted to be eaten either fresh or smoked (Boschung et al. 1983:375-376).

Positive reactions obtained on tools recovered from Locus 1 include one American eel, one gizzard shad, two deer, and two guinea pig, which might indicate exploitation of other rodents, such as beaver, porcupine, and/or a member of the squirrel family. Locus 1 provides the only evidence for the possible use of these rodents. Unfortunately, no soil control samples were examined, and it is not possible to rule out contamination as the source for these positive reactions. Although three soil controls were examined from this locus and none yielded positive reactions, they were not sufficient to identify all possible false positive reactions. Therefore, interpretations of the positive reactions obtained from the tools remain tentative.

B. LOCUS 2

Locus 2 is represented by a jasper late-stage biface (Sample 97/59/25) from EU 218 in Block 18. This artifact yielded negative results to all antisera tested.

C. LOCUS 3

1. Metate Block

A total of 37 artifacts were tested from the Metate block in Locus 3. Sample 510 is a slaty-chert projectile point/flake tool from EU 374 that tested positive to striped bass antiserum. Artifact 974 is a quartz biface from EU 396 that tested positive to Atlantic croaker antiserum. No soil controls specifically associated with these tools were tested. Positive reactions to striped bass and Atlantic croaker antisera might represent use of the tools to hunt/process fish from a member of the Perciformes order, which is the largest order of vertebrates and includes many North American marine and freshwater fishes. This order is divided into 78 families of typical spiny-rayed or percoid fishes (Boschung et al. 1983:532). Families in this order noted in the Delaware Bay include Percichthyidae (temperate bass), Centrarchidae (sunfish), Percidae (perch), and Cottidae (sculpin family) (R. Jacoby, Archaeological Field Supervisor, The Louis Berger Group, Inc.,

personal communication January 18, 1999). Because no soil controls specifically associated with these artifacts were tested, one or both of the positive results might also be due to soil contamination.

Sample 1171.1 is a quartz biface from EU 456 that yielded a positive result to American eel. This biface might have been used by the prehistoric site occupants to process American eel. However, in the absence of a soil control for this artifact, it cannot be ruled out that the positive result is due to soil contamination.

General soil controls from the B-horizon in EUs 353, 370, 436, and 455 of the Metate block were also tested. Soil Sample 316 yielded a positive result to guinea pig antiserum, and Soil Sample 1168 tested positive to gizzard shad antiserum. These positive results either indicate soil contamination from modern animal activity and/or soil compounds such as bacteria, or perhaps represent areas of prehistoric butchering activity.

Sample 98/2/529 is a quartz biface fragment from Feature 96, a cluster of fire-cracked rock in EU 397. This biface fragment tested positive to American eel antiserum. The soil control for this artifact (Sample 98/2/812) yielded negative results to American eel antiserum, suggesting that the biface might have been used to process American eel. The soil control tested positive to deer antiserum, suggesting that historic/modern deer feces might have introduced proteins into the soil in this area.

Sample 98/2/803 is a possible chert drill tip found in EU 424. A positive result to deer antiserum for this artifact may indicate that it was used to process remains from a member of the deer family, such as white-tailed deer. The soil control submitted for this artifact (Sample 98/2/781) was recovered from a lower level in EU 421 and tested negative against all antisera tested. It is possible that deer activity or soil compounds in the vicinity of the artifact, but not the soil control sample, resulted in a false positive reaction for the artifact.

Eleven soil control samples were examined from the Metate block. Some of the soil samples were directly associated with individual artifacts, and others were not. Of these 11 controls, three yielded positive reactions to gizzard shad, deer, and guinea pig. Positive reactions on tools to gizzard shad and guinea pig appear to be false positives that might have been the result of soil contamination. Positive reactions to American eel (two), Atlantic croaker (one), striped bass (one), and deer (one) were obtained on other artifacts, some of which were accompanied by specific soil control samples, and some of which were not. The majority of the positive reactions from the Metate block are to fish, with only a single positive deer result representing mammals. Soil control samples exhibited single instances of rodent, deer, and gizzard shad contamination.

2. *Feature 30*

Ten artifacts were submitted for analysis from the Feature 30 block in Locus 3. Feature 30 is a large pit, with a possible internal lining and stepped sides, that might have been used as a storage pit or burial pit. Sample 757S represents feature fill submitted as a soil control. This sample tested positively to serum from a non-immunized goat (pre-immune serum), indicating non-specific protein interaction not based on immunological specificity of any antibody.

Sample 1379 is a chert scraper found in a large, flat-bottomed pit (Feature 37). Sample 1278 represents feature fill tested as a soil control for the scraper. The soil control yielded negative results to all antisera tested. The scraper yielded a positive result to American eel antiserum, suggesting that it was used to process American eel.

A jasper projectile point (Sample 1352) from EU 472 and a chert projectile point (Sample 1381) from EU 488 both tested positively to gizzard shad antiserum. In the absence of soil controls for these artifacts, it is not

possible to determine whether the positive results indicate that the tools were used to procure gizzard shad or that the soil has been contaminated.

Sample 9913 is a chert biface and Sample 9915 is a jasper biface. Both artifacts were found outside the Feature 30 excavation block. Both of the bifaces and their associated soil controls yielded positive results to deer antiserum, indicating some type of soil contamination, possibly from modern deer activity in the area or soil compounds such as bacteria.

Six soil control samples were examined from the Feature 30 block. Two soil samples yielded positive reactions to deer, and two artifacts yielded positive reactions to deer. Since these two tools were directly associated with the soil controls that tested positively for deer, no interpretation can be made regarding the use of these tools. Other tools yielded evidence that gizzard shad (two) was processed by occupants of the site. In addition, Feature 37 yielded a scraper that tested positively to the American eel antiserum, and the soil control sample for the scraper reacted negatively to the antiserum. These results lead to an interpretation that American eel was probably processed at the site.

3. *Block 3*

Three artifacts and two soil control samples were examined from Block 3 of Locus 3. The artifacts consist of an argillite projectile point, a chert projectile point base recovered from a cluster of fire-cracked rock, and a quartz projectile point base also recovered from a cluster of fire-cracked rock. All three artifacts yielded negative results to all antisera tested.

4. *Block 6*

Samples 97/55/139 and 97/55/535 are bifaces recovered from Block 6 in Locus 3. No soil controls were tested for these artifacts. Sample 97/55/139 is a quartz late-stage biface from EU 84 that yielded positive results to American eel antiserum. A positive result to deer antiserum was obtained for Sample 97/55/535, a quartzite middle-stage biface from a chipping cluster (Feature 4). These tools might have been used to process eel and a member of the Cervidae family, such as white-tailed deer, respectively. Alternatively, one or both of these positive results might be the result of some type of soil contamination.

5. *General Locus 3*

Positive protein residue results were obtained from three jasper projectile points and a jasper middle-stage biface from Locus 3. Sample 219 is a jasper projectile point that tested positively to deer antiserum. This point might have been used to hunt a member of the Cervidae family. However, because no soil control was tested for this artifact, the possibility that the positive result was caused by soil contamination must also be considered.

Sample 98/2/241 is a jasper projectile point from Feature 33, a small, round, basin-shaped pit in EU 337. This point yielded positive results to American eel, bay anchovy, and deer antisera. The associated soil control (Sample 98/2/241S) from this feature also yielded positive results to American eel antiserum. It is possible that the pit was used to process/discard American eel, bay anchovy, and perhaps white-tailed deer remains after the point had been deposited in the pit. The positive reaction of the soil control to American eel antiserum might indicate that soil contamination was the cause of the projectile point's positive reaction to American eel antiserum. The point might have been used to hunt white-tailed deer or possibly another member of the Cervidae family.

Positive reactions to American eel and deer antisera were also obtained from Sample 98/2/260, a jasper middle-stage biface recovered from a diffuse cluster of fire-cracked rock (Feature 35). This tool might have been used to process eel and a member of the Cervidae family. However, in the absence of a soil control, it is not possible to determine whether one or both of these positive results are due to soil contamination.

Sample 98/2/300 is a jasper projectile point that yielded a positive result to bay anchovy and catfish antisera. These results might indicate use of anchovies and a member of the Ictaluridae family (Bullhead catfish). Previous tests have shown that the catfish antiserum will also react positively with common carp proteins (*Cyprinus carpio*), which is an introduced species. It is possible that catfish antiserum will react positively with proteins from other members of the Cyprinidae family (carp and minnows). Because no soil control was tested with this artifact, it is not possible to rule out soil contamination as a cause for one or both positive results from the projectile point.

Only a single soil control sample was examined to assist in interpreting the protein record for these tools. It was associated with a single artifact and yielded evidence that the positive reaction to American eel antiserum might be the result of contamination unless the soil control sample was collected in an area where the fish were processed and blood and fluids were allowed to seep into the soil. Positive reactions recovered from artifacts from the generalized area include American eel, bay anchovy, catfish, and deer. This area might have been used primarily for processing fish.

D. LOCUS 5, FEATURE 99

Feature 99 in Locus 5 has been described as both a parabolic pit and a chipping cluster. A total of eight artifacts from this feature were tested for protein residues, and five of these artifacts yielded positive results. No soil controls were available for any of the artifacts. Sample 98/2/9903 is a jasper projectile point that tested positive to American eel and catfish antisera. These results might indicate utilization of American eel and a member of the Ictaluridae family, or possibly the Cyprinidae family, by the prehistoric site occupants. One or both of these positive results might also have been caused by soil contamination.

A quartz biface (Sample 98/2/9909) tested positive to turkey antiserum. This biface might have been used to process wild turkeys or ducks. Turkeys (*Meleagris gallopavo*) are large birds with strong legs. "They generally walk or run, but can fly strongly for short distances" (Perrins and Middleton 1989:130). Turkeys are found primarily in oak woodlands and pine forests of the eastern and southwestern United States, as well as in mountain forests and broken woodlands of the western United States, south of central Colorado (Peterson 1961:92). The turkey has disappeared from much of its original range. Previous tests have shown that duck blood will also yield a weak positive result to turkey antiserum. Ducks, along with geese and swans, belong to the Anatidae family. Goose and swan blood has not yet been tested against turkey antiserum. The positive result to turkey antiserum may represent utilization of turkey, ducks, or another member of the Anatidae family. It is also possible that this positive result is due to soil contamination.

Sample 98/2/9911 is a jasper biface, and Sample 98/2/9916 is a quartzite flake. Both artifacts tested positively to catfish antiserum. These tools might have been used to process fish from the Ictaluridae family or possibly a member of the Cyprinidae family. In the absence of soil controls, soil contamination must also be considered a possibility.

Sample 98/2/9914 is a chert projectile point that tested positively to deer and weakfish antisera. The point might have been used to hunt a member of the Cervidae family and to catch weakfish (*Cynoscion regalis*). One or both of these positive results might also be the result of soil contamination.

Protein residue analysis from Feature 99 in Locus 5 yielded more evidence of catfish (and related fish) than any other area of the site. In addition, single positive reactions to American eel, weakfish, deer, and turkey antisera were recovered. This feature provides the only evidence for the possible use of turkey at the site. Although no soil control samples were examined from this feature or locus to assist in interpreting the protein record, the pattern of positive protein recovery differs from other areas. Soil control samples would have been very helpful in increasing confidence of these interpretations.

IV. SUMMARY AND CONCLUSIONS

Protein residue analysis of 83 lithic artifacts from the Puncheon Run Site (7K-C-51) yielded a total of 25 positive results. The remaining artifacts yielded negative results to all antisera tested. As for those artifacts yielding only negative results, it is possible that they were not used to hunt/process fish or animal resources; they were used to hunt/process animals, fish, and avian species other than those represented by the 13 antisera tested; and/or that insufficient amounts of proteins were retained on the artifact surfaces.

Animal resources that might have been utilized at this site include white-tailed deer and/or possibly another member of the Cervidae family; a rodent such as beaver, porcupine, and/or a member of the Sciuridae (squirrel) family; and wild turkey or possibly a member of the Anatidae family, such as duck. The riverine subsistence might have included American eel, gizzard shad, anchovies, weakfish, a member of the Perciformes order, and a member of the Ictaluridae family and/or possibly a member of the Cyprinidae family. Lack of associated soil controls for each artifact leads to serious problems in interpreting the positive results for the artifacts. Because soil controls were not available for each artifact tested, positive results cannot be considered conclusive evidence of use. Positive results might be the result of contaminants, such as soil bacteria or animal feces. Of the five positive reactions obtained on soil control samples from the site, two were positive to deer, one to guinea pig, and two to fish (gizzard shad and American eel). Comparison of positive results recovered from artifacts with those from soil control samples (Table J-4) suggests that guinea pig-type (rodent) protein in the soil or compounds such as bacteria or iron chlorates that are being recognized by the guinea pig antiserum are a problem contaminant. Contamination from deer, American eel, and gizzard shad proteins and/or soil compounds that are recognized by these antisera appears to occur at lower frequencies.

Table J-1: Antisera Used in Testing Artifacts From the Puncheon Run Site

Antisera	Source	Possible Positive Result Interpretations
<i>Fish:</i>		
American eel	Robert Sargeant	<i>Anguilla rostrata</i> (American eel)
Atlantic croaker	Robert Sargeant	Perciformes order (Spiny-rayed [percoid] fishes)
Bay anchovy	Robert Sargeant	Engraulidae family (Anchovies)
Catfish	Sigma Chemical Company	Ictaluridae family (Bullhead catfish); <i>Cyprinus carpio</i> (Common carp); and probably other members of the Cyprinidae family (carp and minnows)
Gizzard shad	Robert Sargeant	<i>Dorosoma cepedianum</i> (Gizzard shad)
Striped bass	Robert Sargeant	Perciformes order (Spiny-rayed [percoid] fishes)
Trout	Sigma Chemical Company	Salmonidae family (Trout and Salmon)
Weakfish	Robert Sargeant	<i>Cynoscion regalis</i> (Weakfish)
<i>Animals:</i>		
Bear	ICN Pharmaceuticals, Inc.	Ursidae (Bear family)
Deer	ICN Pharmaceuticals, Inc.	Cervidae (Deer family)
Guinea pig	Sigma Chemical Company	<i>Castor</i> sp. (Beaver); <i>Erethizon dorsatum</i> (Porcupine); Sciuridae (Squirrel family)
Rabbit	Sigma Chemical Company	Leporidae family (Rabbits and Hares)
Turkey	Sigma Chemical Company	<i>Meleagris gallopavo</i> (Turkey); Anatidae (Duck family)

Table J-2: Provenience Data for Samples from the Puncheon Run Site

Cat. No.	Locus	Area	Unit	Stratum/ Level	Feature No.	Description	Analysis
98/2/1144	1	Silo Pit	.	C-7	64	Chert projectile point from possible cultural flat basin pit	Protein residue
98/2/1138	1	Silo Pit	.	C-7	64	Soil control for chert point (No. 98/2/1144)	Protein residue
98/2/1344	1	Silo Pit	.	A	60	Jasper point	Protein residue
98/2/36.1	1	BPZ	257	C-3	.	Quartz middle-stage biface	Protein residue
98/2/36.2	1	BPZ	257	C-3	.	Chert projectile point	Protein residue
97/58/38	1	BPZ	195	C-3	.	Jasper late-stage biface	Protein residue
98/2/40	1	BPZ	257	C-4	.	Chert late-stage biface	Protein residue
98/2/51	1	BPZ	259	C-3	.	Quartz middle-stage biface	Protein residue
98/2/79	1	BPZ	266	C-3	.	Jasper projectile point	Protein residue
97/58/102	1	BPZ	197	B-3	.	Quartz middle-stage biface	Protein residue
98/2/344	1	BPZ	361	C-3	.	Jasper projectile point	Protein residue
98/2/344S	1	BPZ	361	C-3	.	Soil control for jasper point (#98/2/344)	Protein residue
98/2/356	1	BPZ	360	B-2	.	Chert projectile point	Protein residue
98/2/356S	1	BPZ	360	B-2	.	Soil control for chert point (#98/2/356)	Protein residue
98/2/417	1	BPZ	343	C-3	.	Chert middle-stage biface	Protein residue
98/2/431	1	BPZ	384	C-3	.	Rhyolite projectile point	Protein residue
98/2/436	1	BPZ	378	C-3	.	Quartz middle-stage biface	Protein residue
98/2/877	1	BPZ	392	C-3	.	Rhyolite biface fragment	Protein residue
97/59/25	2	Block 18	218	B-2	.	Jasper late-stage biface	Protein residue
360	3	Metate Block	370	B-2	.	Hammer/grinding stone	Protein residue
470.1	3	Metate Block	382	B-2	.	Jasper projectile point	Protein residue
470.2	3	Metate Block	382	B-2	.	Metasedimentary projectile point	Protein residue

Table J-2 (continued)

Cat. No.	Locus	Area	Unit	Stratum/ Level	Feature No.	Description	Analysis
543	3	Metate Block	403	B-2	.	Chert projectile point; basal cortex?	Protein residue
543S	3	Metate Block	403	B-2	.	E-horizon; Soil control	Protein residue
545	3	Metate Block	404	B-2	.	Quartz biface	Protein residue
602.1	3	Metate Block	405	B-2	.	Chert biface	Protein residue
602.2	3	Metate Block	405	B-2	.	Biface	Protein residue
602S	3	Metate Block	405	B-2	.	E-horizon; Soil control	Protein residue
604	3	Metate Block	407	B-2	.	Jasper projectile point	Protein residue
924	3	Metate Block	430	B-2	.	Chert projectile point	Protein residue
942	3	Metate Block	437	B-2	.	Quartzite biface	Protein residue
1027	3	Metate Block	449	B-2	.	Jasper projectile point; irregular	Protein residue
213	3	Metate Block	331	B-3	.	Chert projectile point; basal cortex	Protein residue
315	3	Metate Block	353	B-3	.	Jasper projectile point	Protein residue
432	3	Metate Block	407	B-3	.	Jasper projectile point; irregular	Protein residue
510	3	Metate Block	374	B-3	.	Slaty-chert projectile point; flake tool	Protein residue
796	3	Metate Block	423	B-3	.	Jasper scraper	Protein residue
849.1	3	Metate Block	356	B-3	.	Jasper projectile point; basal cortex	Protein residue
849.2	3	Metate Block	356	B-3	.	Jasper projectile point; basal cortex	Protein residue
911.1	3	Metate Block	387	B-3	.	Jasper projectile point	Protein residue
911.2	3	Metate Block	387	B-3	.	Jasper projectile point	Protein residue
953	3	Metate Block	362	B-3	.	Jasper projectile point; basal cortex	Protein residue
974	3	Metate Block	396	B-3	.	Quartz biface	Protein residue

Table J-2 (continued)

Cat. No.	Locus	Area	Unit	Stratum/ Level	Feature No.	Description	Analysis
994	3	Metate Block	441	B-3	.	Jasper biface	Protein residue
1171.1	3	Metate Block	456	B-3	.	Quartz biface	Protein residue
1171.2	3	Metate Block	456	B-3	.	Quartzite biface	Protein residue
316	3	Metate Block	353	B-3	.	B-horizon; Soil control	Protein residue
940	3	Metate Block	370	B-3	.	B-horizon; Soil control	Protein residue
979	3	Metate Block	436	B-3	97	B-horizon; Soil control from FCR* cluster	Protein residue
1168	3	Metate Block	455	B-3	97	B-horizon; Soil control from FCR cluster	Protein residue
781	3	Metate Block	421	B-4	.	Jasper projectile point	Protein residue
781S	3	Metate Block	421	B-4	.	B-horizon; Soil control for jasper projectile point	Protein residue
1004	3	Metate Block	442	B-4	.	Jasper projectile point; basal cortex	Protein residue
1172	3	Metate Block	455	B-4	.	Quartz biface	Protein residue
831	3	Metate Block	427	B-5	.	Quartz projectile point; basal cortex	Protein residue
1000	3	Metate Block	441	B-5	.	Jasper projectile point	Protein residue
98/2/289	3	Metate Block	348	B-2	.	Quartz biface fragment	Protein residue
98/2/529	3	Metate Block	397	B-2	96	Quartz biface fragment from FCR cluster	Protein residue
98/2/812	3	Metate Block	397	B-3	96	Soil control for quartz biface (#98/2/529) from FCR cluster	Protein residue
98/2/803	3	Metate Block	424	B-2	.	Chert? drill tip	Protein residue
98/2/781	3	Metate Block	421	B-4	.	Soil control for chert? drill tip (#98/2/803)	Protein residue
98/2/823	3	Metate Block	427	B-2	.	Quartz biface fragment	Protein residue
98/2/840	3	Metate Block	410	B-3	.	Soil control for quartz biface fragment (#98/2/823)	Protein residue
98/2/1039	3	Metate Block	449	B-4	.	Quartz middle-stage biface	Protein residue

Table J-2 (continued)

Cat. No.	Locus	Area	Unit	Stratum/ Level	Feature No.	Description	Analysis
98/2/979	3	Metate Block	436	B-3	.	Soil control for quartz middle- stage biface (#98/2/1039)	Protein residue
98/2/1197	3	Metate Block	459	B-4	.	Jasper early-stage biface	Protein residue
756	3	Feature 30 Block	318	A-2	30	Quartz biface with transverse break from large storage or burial pit	Protein residue
757	3	Feature 30 Block	318	A-3	30	Chert projectile point from large storage or burial pit	Protein residue
757S	3	Feature 30 Block	318	A-3	30	Feature fill; Soil control from large storage or burial pit	Protein residue
193	3	Feature 30 Block	320	A-3	30	Jasper scraper from large storage or burial pit	Protein residue
98/2/663	3	Feature 30 Block	321	A-5	30	Argillite biface base from large storage or burial pit	Protein residue
98/2/663S	3	Feature 30 Block	321	A-5	30	Soil control for argillite biface base (#98/2/663)	Protein residue
98/2/1367	3	Feature 30 Block	482	B-2	30	Quartz biface fragment from large storage or burial pit	Protein residue
520	3	Feature 30 Block	393	A-3	38	Jasper projectile point from large storage or burial pit	Protein residue
937	3	Feature 30 Block	432	A-3	38	Feature fill; Soil control from large storage or burial pit	Protein residue
1379	3	Feature 30 Block	473	A-3	37	Chert scraper from large, flat- bottomed pit	Protein residue
1278	3	Feature 30 Block	468	A-3	37	Feature fill; Soil control from large, flat-bottomed pit	Protein residue
456	3	Feature 30 Block	368	B-2	.	Chert biface with transverse break	Protein residue
1352	3	Feature 30 Block	472	B-2	.	Jasper projectile point	Protein residue
1381	3	Feature 30 Block	488	B-3	.	Chert projectile point	Protein residue
498	3	Feature 30 Block	315	C-3	.	Rhyolite projectile point	Protein residue
9913	3	Feature 30 Block	.	.	.	Chert biface found outside excavation block	Protein residue
9913S	3	Feature 30 Block	.	.	.	Soil control for chert biface	Protein residue
9915	3	Feature 30 Block	.	.	.	Jasper biface	Protein residue
9915S	3	Feature 30 Block	.	.	.	Soil control for jasper biface	Protein residue

Table J-2 (continued)

Cat. No.	Locus	Area	Unit	Stratum/ Level	Feature No.	Description	Analysis
97/55/98	3	Block 3	68	B-2	.	Argillite projectile point	Protein residue
97/55/342	3	Block 3	134	B-2	16a	Chert projectile point base from FCR cluster	Protein residue
97/55/516	3	Block 3	134	B-2	16a	Soil control for chert projectile point base (#98/55/342)	Protein residue
97/55/345	3	Block 3	137	B-2	16b	Quartz projectile point base from FCR cluster	Protein residue
97/55/518	3	Block 3	137	B-2	16b	Soil control for quartz projectile point base (#97/55/345)	Protein residue
97/55/139	3	Block 6	84	B-3	.	Quartz late-stage biface	Protein residue
97/55/535	3	Block 6	490	B-2	4	Quartzite middle-stage biface from chipping cluster	Protein residue
188	3	Quartz projectile point	Protein residue
219	3	Jasper projectile point	Protein residue
98/2/241	3	.	337	A-2	33	Jasper projectile point from small pit	Protein residue
98/2/241S	3	.	337	A-2	33	Soil control for jasper point (#98/2/241)	Protein residue
98/2/260	3	.	345	B-2	35	Jasper middle-stage biface from diffuse FCR cluster	Protein residue
98/2/300	3	.	352	B-2	.	Jasper projectile point	Protein residue
98/2/9903	5	Keith	.	A-2	99	Jasper point from chipping cluster	Protein residue
98/2/9906	5	Keith	.	A-2	99	Chert biface from chipping cluster	Protein residue
98/2/9908	5	Keith	.	A-2	99	Chert projectile point from chipping cluster	Protein residue
98/2/9909	5	Keith	.	A-3	99	Quartzite biface from chipping cluster	Protein residue
98/2/9911	5	Keith	.	A-3	99	Jasper biface from chipping cluster	Protein residue
98/2/9913	5	Keith	.	A-4	99	Chert biface from chipping cluster	Protein residue
98/2/9914	5	Keith	.	A-4	99	Chert projectile point from chipping cluster	Protein residue
98/2/9916	5	Keith	.	A-4	99	Quartzite flake from chipping cluster	Protein residue

* FCR=Fire-cracked rock

Table J-3: Positive Results for Samples From the Puncheon Run Site

Sample No.	Description	Positive Result (Antiserum Type)	Possible Animal(s) Represented
<i>Locus 1</i>			
98/2/1144	Chert projectile point	Deer	Cervidae (Deer family)
98/2/36.2	Chert projectile point	Guinea pig	<i>Castor</i> sp. (Beaver); <i>Erethizon dorsatum</i> (Porcupine); Sciuridae (Squirrel family); or soil contamination
97/58/38	Jasper late-stage biface	Guinea pig	<i>Castor</i> sp. (Beaver); <i>Erethizon dorsatum</i> (Porcupine); Sciuridae (Squirrel family); or soil contamination
		Gizzard shad	<i>Dorosoma cepedianum</i> (Gizzard shad) or soil contamination
98/2/79	Jasper projectile point	Deer	Cervidae (Deer family) or soil contamination
98/2/356	Chert projectile point	American eel	<i>Anguilla rostrata</i> (American eel)
<i>Metate Block, Locus 3</i>			
510	Slatey-chert projectile point; flake tool	Striped bass	Perciformes order or soil contamination
974	Quartz biface	Atlantic croaker	Perciformes order or soil contamination
1171.1	Quartz biface	American eel	<i>Anguilla rostrata</i> (American eel) or soil contamination
316	B-horizon soil control	Guinea pig	Modern rodent activity or other soil contamination
1168	B-horizon soil control	Gizzard shad	Soil contamination
98/2/529	Quartz biface fragment	American eel	<i>Anguilla rostrata</i> (American eel)
98/2/812	Soil control for quartz biface fragment (#98/2/529)	Deer	Soil contamination
98/2/803	Possible chert drill tip	Deer	Cervidae (Deer family) or soil contamination
<i>Feature 30 Block, Locus 3</i>			
757S	Feature 30 fill; soil control	Pre-immune goat serum	Non-specific protein interaction not based on immunological specificity of the antibody
1379	Chert scraper	American eel	<i>Anguilla rostrata</i> (American eel)
1352	Jasper projectile point	Gizzard shad	<i>Dorosoma cepedianum</i> (Gizzard shad) or soil contamination
1381	Chert projectile point	Gizzard shad	<i>Dorosoma cepedianum</i> (Gizzard shad) or soil contamination
9913	Chert biface found outside excavation block	Deer	Soil contamination
9913S	Soil control for chert biface	Deer	Soil contamination
9915	Jasper biface found outside excavation block	Deer	Soil contamination
9915S	Soil control for jasper biface	Deer	Soil contamination

Table J-3 (continued)

Sample No.	Description	Positive Result (Antiserum Type)	Possible Animal(s) Represented
<i>Block 6, Locus 3</i>			
97/55/139	Quartz late-stage biface	American eel	<i>Anguilla rostrata</i> (American eel) or soil contamination
97/55/535	Quartzite middle-stage biface	Deer	Cervidae (Deer family) or soil contamination
<i>Locus 3</i>			
219	Jasper projectile point	Deer	Cervidae (Deer family) or soil contamination
98/2/241	Jasper projectile point	American eel	Soil contamination
		Bay anchovy	Engraulidae family (Anchovies)
		Deer	Cervidae (Deer family)
98/2/241S	Soil control for jasper projectile point	American eel	Soil contamination
<i>Locus 3</i>			
98/2/260	Jasper middle-stage biface	American eel	<i>Anguilla rostrata</i> (American eel) or soil contamination
		Deer	Cervidae (Deer family) or soil contamination
98/2/300	Jasper projectile point	Bay anchovy	Engraulidae family (Anchovies) or soil contamination
		Catfish	Ictaluridae family (Bullhead catfish); Cyprinidae (Carp and minnows); or soil contamination
<i>Feature 99, Locus 5</i>			
98/2/9903	Jasper projectile point	American eel	<i>Anguilla rostrata</i> (American eel) or soil contamination
		Catfish	Ictaluridae family (Bullhead catfish); Cyprinidae (Carp and minnows); or soil contamination
98/2/9909	Quartzite biface	Turkey	<i>Meleagris gallopavo</i> (Turkey); Anatidae (Duck family); or soil contamination
98/2/9911	Jasper biface	Catfish	Ictaluridae family (Bullhead catfish); Cyprinidae (Carp and minnows); or soil contamination
98/2/9916	Quartzite flake	Catfish	Ictaluridae family (Bullhead catfish); Cyprinidae (Carp and minnows); or soil contamination
98/2/9914	Chert projectile point	Deer	Cervidae (Deer family) or soil contamination
		Weakfish	<i>Cynoscion regalis</i> (Weakfish) or soil contamination

Table J-4: Comparison of Positive Reactions for Artifacts and Soil Controls from the Puncheon Run Site

Antisera	Total Artifacts with Positive Results	Locus 1	Locus 3				Locus 5	Soil Controls with Positive Results
			Metate Block	F. 30 Block	Block 6	General		
<i>Fish:</i>								
American eel	8	1	2	1	1	1+1*	1	1
Atlantic croaker	1	.	1
Bay anchovy	2	2	.	.
Catfish	4	1	3	.
Gizzard shad	3	1	.	2	.	.	.	1
Striped bass	1	.	1
Weakfish	1	1	.
<i>Animal:</i>								
Deer	10	2	1	2*	1	3	1	2
Guinea pig	2	2	1
Turkey	1	1	.
Soil controls examined	None	3	3	3	None	1	None	.

* Indicates a positive result directly associated with soil control contamination

REFERENCES CITED

- Boschung, Herbert T., Jr., James D. Williams, Daniel W. Gotshall, David K. Caldwell, and Melba C. Caldwell
1983 *The National Audubon Society Field Guide to North American Fishes, Whales & Dolphins*. Alfred A. Knopf, New York.
- Culliford, Brian J.
1964 Precipitation Reactions in Forensic Problems. *Nature* 201:1092-1094.
- 1971 *The Examination and Typing of Bloodstains in the Crime Laboratory*. United States Department of Justice, United States Government Printing Office, Stock 2700-0083, Washington, D.C.
- Gaensslen, R.E.
1983 *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. United States Department of Justice, Washington, D.C.
- Newman, M., and P. Julig
1989 The Identification of Protein Residues on Lithic Artifacts from a Stratified Boreal Forest Site. *Canadian Journal of Archaeology* 13:119-132.
- Newman, Margaret E., Robert M. Yohe, Howard Ceri, and Mark Q. Sutton
1993 Immunological Protein Residue Analysis of Non-lithic Archaeological Materials. *Journal of Archaeological Science* 20:93-100.
- Perrins, Christopher M., and Alex L.A. Middleton (editors)
1989 *The Encyclopedia of Birds*. Facts On File, New York.
- Peterson, Roger Tory
1961 *A Field Guide to Western Birds*. Houghton Mifflin Co., Boston.
- Whitaker, John O., Jr.
1980 *The Audubon Society Field Guide to North American Mammals*. Alfred A. Knopf, Inc., New York.